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Comparisons of Single-Species, Microcosm and Experimental Pond Responses to Atrazine Exposure

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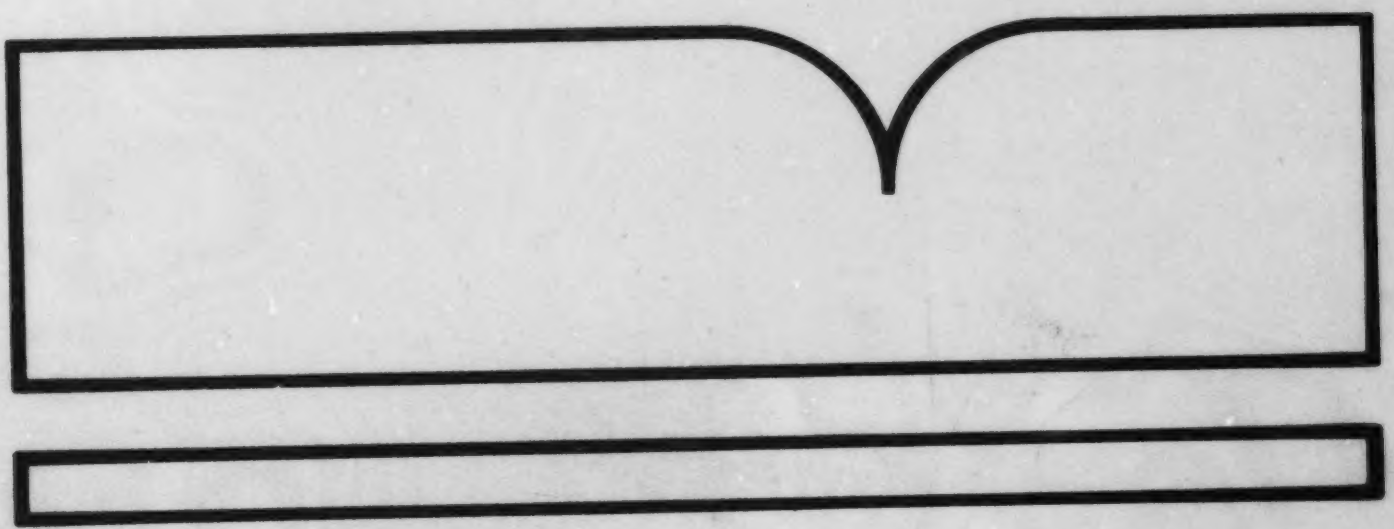

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16. ABSTRACT

Results from single-species toxicity tests form the basis for guidance about concentrations of chemicals that are likely to be environmentally hazardous. Microcosm bioassays have been proposed as better tools for this purpose under some circumstances. Rarely have comparisons been made among the responses in the various test systems and those seen in natural communities. We compared the responses among single-species algal assays, a synthetic microcosm (the Taub microcosm) and experimental ponds exposed to similar concentrations of atrazine by comparing concentrations of atrazine that reduced algal activity or biomass to 50% of control values(EC50). Eight algal species were examined; mean species EC50 values were 103 to 159ug/L for ^{14}C uptake ranged from 37 to 308 ug/L. For the Taub microcosm, EC50 values were 103 to 159 ug/L for ^{14}C uptake, 126 to 165 ug/L for dissolved oxygen production and 106 to 164 ug/L for dissolved oxygen consumption. Values were dependent on the time intervals selected for calculations. In the ponds, EC50 values for ^{14}C uptake were 100 ug/L and for chlorophyll a, 82 ug/L. The basic similarity among EC50 values across test systems suggested that results from a combination of single-species assays, or the Taub microcosm, provided a reasonable estimate of the concentration of atrazine that produced similar direct effects on more natural communities.

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COMPARISONS OF SINGLE-SPECIES, MICROCOSM AND EXPERIMENTAL POND RESPONSES TO ATRAZINE EXPOSURE

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Abstract—Results from single-species toxicity tests form the basis for guidance about concentrations of chemicals that are likely to be environmentally hazardous. Microcosm bioassays have been proposed as better tools for this purpose under some circumstances. Rarely have comparisons been made among the responses in the various test systems and those seen in natural communities. We compared the responses among single-species algal assays, a synthetic microcosm (the Taub microcosm) and experimental ponds exposed to similar concentrations of atrazine by comparing concentrations of atrazine that reduced algal activity or biomass to 50% of control values (EC50). Eight algal species were examined; mean species EC50 values for ^{14}C uptake ranged from 37 to 308 μg/L. For the Taub microcosm, EC50 values were 103 to 159 μg/L for ^{14}C uptake, 126 to 165 μg/L for dissolved oxygen production and 106 to 164 μg/L for dissolved oxygen consumption. Values were dependent on the time intervals selected for calculations. In the ponds, EC50 values for ^{14}C uptake were 100 μg/L and for chlorophyll *a*, 82 μg/L. The basic similarity among EC50 values across test systems suggests that results from a combination of single-species assays, or the Taub microcosm, provided a reasonable estimate of the concentration of atrazine that produced similar direct effects on more natural communities.

Keywords—Atrazine Microcosm Bioassays Field ponds

INTRODUCTION

Evaluation of the potential environmental toxicity of chemicals relies primarily on results obtained from single-species toxicity tests conducted under standardized laboratory conditions. These single-species tests are intended to signal the concentrations at which potentially toxic chemicals are likely to be environmentally hazardous. National criteria that establish safe levels for selected chemicals are calculated from the results of an array of single-species toxicity tests [1]. Initial phases of proposed hazard assessment methods incorporate results from even fewer single-species tests [2,3].

One shortcoming of the use of single-species tests is that they are ecologically unrealistic since they do not incorporate the complexity of assemblages of organisms interacting with each other and with their abiotic environment [4–7]. Various kinds of tests have been developed to try to incorporate species interactions [8–10]. These include tests designed to assess effects on specific kinds of interactions and usually include a few interacting species, often only two. For example, a pair of competitors, or a predator and a prey species might be used. They also include microcosm tests that incorporate interspecies interactions, but effects on specific kinds of interactions are not generally measured. Instead, effects on the outcome of the multiplicity of interactions are measured by population shifts, community structural changes or by measures such as biomass, productivity, respiration or nutrient processing.

Research to verify results from test systems by comparison with results from natural systems experimentally or accidentally perturbed generally compares site-specific systems. For example, microcosm results have been compared with results from the specific systems they are intended to simulate [11–13]. Responses from single-species laboratory assays have been compared with responses of the same species under more natural conditions stressed by the same chemical [14–18]. Few com-

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parisons have been made using generic microcosm systems, i.e., systems that are not designed to mimic any specific natural aquatic ecosystem. Comparisons have been mainly qualitative. Even though there are few comparisons between test systems and the natural environment, most toxicity screening relies on generic tests during most phases of hazard evaluation.

This article reports results of a study designed to determine how well standardized, generic tests estimate the concentration of a chemical that produces similar effects in more nearly natural situations. It compares responses of several single-species tests, a generic microcosm and experimental ponds to exposure to the same chemical, atrazine. We hope that by making these kinds of comparisons we can begin to infer how well generic test systems signal harm to ecosystems. If we conduct a series of tests and find that our test systems provide a reasonable estimate of effect concentrations, then we gain confidence in their use. However, if results from the test systems inadequately signal harm, then these test systems must be redesigned. This study is only one such comparison, and more are needed.

MATERIALS AND METHODS

General approach

This study compared the responses of algae tests, a generic microcosm (the Taub microcosm) [19] and experimental ponds exposed to similar concentrations of the commonly used herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). Atrazine inhibits photosynthesis by blocking electron transport within the Hill reaction of photosystem II [20]. It is widely used in the midwestern United States to control pest plant species that invade corn and soybean fields [21].

For the purposes of comparing responses across systems, we wanted a measure that was directly comparable among systems, that related directly to the mode of action of the chemical and that was easily and relatively precisely estimated. If generic test systems could not produce comparable results and results that were similar to field results, their usefulness would be severely limited. Because atrazine is a chemical with a specific photosynthetic mode of action, we used measures related to photosynthetic activity to make comparisons across systems. We chose the concentration that reduced activity by 50% (EC50) as the common endpoint because it is easily calculated from dose-response curves. Also, the EC50 occurs toward the center of a dose-response curve, and values toward the center of a regression are more precisely estimated than those toward the extremes (e.g., an EC20 or EC90). Other reports will deal more specifically with doses at which direct and indirect effects were detectable among the different test systems (de-Noyelles, manuscript in preparation; Stay, manuscript in preparation).

For the single-species algal assays, we used ^{14}C uptake after 24 h of exposure to atrazine. For the Taub microcosm, ^{14}C uptake, and net productivity and respiration measured by diel changes in dissolved oxygen (DO) were used. Microcosm chlorophyll *a* was not used to estimate EC50 values because chlorophyll *a* increased at the low exposures to atrazine (see Results). For the ponds, ^{14}C uptake and chlorophyll *a* were used. Diel changes in DO were not used to generate dose-response curves for the ponds because they were very small and subject to large relative variation.

Single-species algal assays

The uptake of ^{14}C after 24 h of exposure to atrazine was used to determine the responses of eight different algal species. All algal species used in these experiments also were used in Taub microcosms (Table 1). Algae were inoculated to an initial concentration of 10^4 cells/ml into 150 ml of Taub and Dollar [22] or algal assay procedure [23] media in 500-ml Erlenmeyer flasks containing various concentrations of atrazine. Cultures were incubated on shakers (100 rpm) under continuous cool-white illumination (400 ft-c) at 24 ± 2°C. After 24 h of exposure, 9-ml subsamples were withdrawn, spiked with 0.5 μCi ^{14}C as $H^{14}CO_3^-$ and incubated in 10-ml capped test tubes for 2 h under the same light and temperature conditions. After incubation, samples were filtered through 0.45-μm membrane filters (Millipore, type HA); the wet filters were dissolved in 7 ml Aquasol®, and ^{14}C activity on the filters was counted with a liquid scintillation counter (Packard Tricarb Model 460C Liquid Scintillation System). Counts were corrected for dark ^{14}C uptake, and counting efficiency was determined with internal spikes.

Taub microcosms

The Taub microcosm was developed as a generic multispecies system that consisted of several interacting trophic levels contained in a relatively small space. It was assembled from 10 algal species, two protozoans, an amphipod, an ostracod, a daphnid, and an uninvited assortment of heterotrophs (bacteria, fungi). These were added to 3 L of synthetic medium [22] over a layer

Table 1. Single-species algal EC50 values based on ^{14}C uptake after 24 h of exposure to atrazine

Alga (species)	Media	LCL (μg/L)	EC50 (μg/L)	UCL (μg/L)
Selenastrum capricornutum	TD	43	53	64
	TD	29	34	40
	AAM	37	42	46
Ankistrodesmus sp.	TD	62	72	82
	TD	54	61	68
	AAM	192[a]	219[a]	257[a]
Chlamydomonas reinhardi	TD	36	48	58
	TD	10	19	28
	TD	38	44	51
Scenedesmus obliquus	TD	27	38	50
	TD	51	57	64
	TD	44	49	55
Chlorella vulgaris	TD	298	325	357
	TD	277	305	334
	AAM	273	293	314
Anabaena cylindrica	TD	173	253	483
	TD	130	178	260
	AAM	140	182	268
Stigeoclonium tenue[b]	TD	108	127	151
	TD	195	224	265
Ulothrix subconstricta[b]	TD	60	88	132

Lower (LCL) and upper (UCL) 95% confidence limits are given. Experiments were run using Taub and Dollar (TD) [22] or algal assay media (AAM).
[a]Suspected outlier.
[b]Tentative species identification.

of silica sand (200 g) enriched with 0.5 g ground chitin and 0.5 g ground cellulose in 3.7 L wide-mouth pickle jars. The community was allowed to adjust for 7 d before a toxicant was added, then monitored for approximately 2 months following the detailed protocol provided by Taub [24,25]. Dose-response data for ^{14}C uptake, chlorophyll *a* and changes in DO are reported here; analytical methodology is reported by Stay et al. [24] and Taub and Read [25]. Nominal atrazine concentrations were 0, 60, 100, 200, 500, 1,000 and 5,000 μg/L. Tests were monitored three times during the experiment and the measured values were used for the dose-response calculations.

Experimental ponds

Experimental ponds were located at the University of Kansas Nelson Environmental Studies area near Lawrence, Kansas. These ponds are 0.045 ha in surface area and are filled to a depth of 2 m (volume equals 470 m^3) with natural water and plankton from an adjacent 0.33-ha reservoir maintained by well water [26].

Two series of experiments were conducted. One, initiated in July 1979, consisted of examining responses to exposures of 20 and 500 μg/L atrazine over a single growing season. The second, initiated in June 1981, consisted of a 3-year study at exposures of 20, 100 and 200 μg/L. During the 3-year period, atrazine levels were returned to near nominal values during late May of the second and third years and, in two of the 20 μg/L ponds, atrazine was raised to 500 μg/L. Atrazine concentrations in the water were monitored biweekly or monthly to determine actual exposure levels.

The pond biota derived from the reservoir were supplemented by addition of several fish species. During the 1979 study, these included bluegill (*Lepomis macrochirus*; 50 fish, average length 8.5 cm), channel catfish (*Ictalurus punctatus*; 20 fish, average length 11.6 cm) and gizzard shad (*Dorosoma cepedianum*; 7 fish, average length 18.5 cm) [26]. During 1981, these species were stocked at densities of 50 (average length 4.5 cm), 20 (average length 12.5 cm) and 10 (average length 35 cm) per pond, respectively. In addition, grass carp, (*Ctyenopharyngodon idella*; 4 fish, average length 20.5 cm) were added to the ponds. Exclosures

excluded grass carp from 10% of each pond. A natural invasion of macrophytes occurred in these experimental ponds.

The ^{14}C uptake data derived from the 1979 study (for methods, see ref. 26) and ^{14}C uptake and chlorophyll *a* from the first year of the 1981–1983 study were used to generate the dose-response curves and estimate EC50 values.

Experiments were also conducted to examine the short-term response of the algal communities to added atrazine (24-h inhibition). In these experiments, samples collected from control ponds at various times of the year were exposed to several levels of atrazine in the presence of ^{14}C, as $NaH^{14}CO_3$, for 24 h. The uptake of ^{14}C over 24 h was used as the response variable to generate the short-term dose-response curves.

EC50 estimates

We estimated EC50 values from linear regressions of response on $\log_e$ dose over the portion of the dose-response curve where the greatest change in inhibition occurred. We also estimated confidence intervals (95%) around the EC50 values. Regression analyses generate confidence intervals around dependent variables, or in this case, around the response. The response confidence intervals were estimated for the concentration of atrazine that produced 50% inhibition [27]. Then these response confidence intervals were translated into dose confidence intervals [28]. Confidence intervals are reported to give a general measure of precision of each test and to indicate that the results of some tests clearly differ from the results of other tests.

RESULTS

Algal assays

Table 1 summarizes EC50 values along with the corresponding 95% confidence intervals for eight algal species tested. Five species were characterized by similar, relatively low EC50s (<100 μg/L), and three with significantly higher values (127 to 325 μg/L). The range of sensitivity was nearly 10-fold, with *Chlamydomonas* the most sensitive (mean EC50 = 37 μg/L) and *Chlorella* the least sensitive (mean EC50 = 308 μg/L).

Microcosms

^{14}C uptake. The uptake of ^{14}C decreased immediately in microcosms treated with atrazine; recovery began after 10 d in microcosms treated with 60 and 100 μg/L. Slight recovery occurred at 200 μg/L, but, in microcosms treated at higher doses, ^{14}C uptake remained depressed throughout the experiment [24].

Dose-response relationships were constructed and EC50 values were calculated for three time intervals. The first interval (days 11 to 21) consisted of the 10 d immediately after treatment; the second (days 53 to 60) consisted of the last week of the experiment and the third (days 11 to 60) covered the entire experimental period. A typical dose-response relationship is shown in Figure 1, covering days 11 to 60. EC50 values for the three intervals are given in Table 2. The EC50 was lowest for the initial interval (103 μg/L), highest for the final interval (159 μg/L) and intermediate (131 μg/L) for the entire period.

Chlorophyll a. Atrazine apparently stimulated the production of chlorophyll *a* in microcosms exposed to the lower atrazine concentrations, 60 to 200 μg/L (Fig. 2). At the higher concentrations of atrazine, only minimal inhibition occurred; significant reduction in chlorophyll *a* occurred only at the 1,000 and 5,000 μg/L treatment levels after prolonged exposure, seen in the time-averaged values for the final week of the microcosm experiments (Fig. 2). No dose-response relationships were generated for chlorophyll *a* because treatment values were rarely below control values.

The increases in chlorophyll *a* observed in this study are consistent with those reported elsewhere. Cunningham et al. [29] reported up to a fivefold increase in chlorophyll *a* in *Potamogeton perfoliatus* exposed to 130 and 1,200 μg/L atrazine for approximately 1 month, even though photosynthesis was reduced significantly. They suggested that this response is analagous to that seen when aquatic plants are exposed to low light levels.

Table 2. Estimates of Taub microcosm EC50 values using ^{14}C uptake, dissolved oxygen (DO) production (12-h light period) and DO consumption (12-h dark period) over three time intervals

Variable	Interval (d)	LCL (μg/L)	EC50 (μg/L)	UCL (μg/L)
^{14}C uptake	11–21	41	103	254
	53–60	91	159	267
	11–60	90	131	192
Δ DO light	11–21	67	126	238
	53–60	59	154	421
	11–60	121	165	224
Δ DO dark	11–21	65	106	172
	53–60	123	164	222
	11–60	106	142	189

LCL and UCL, lower and upper 95% confidence limits, respectively.

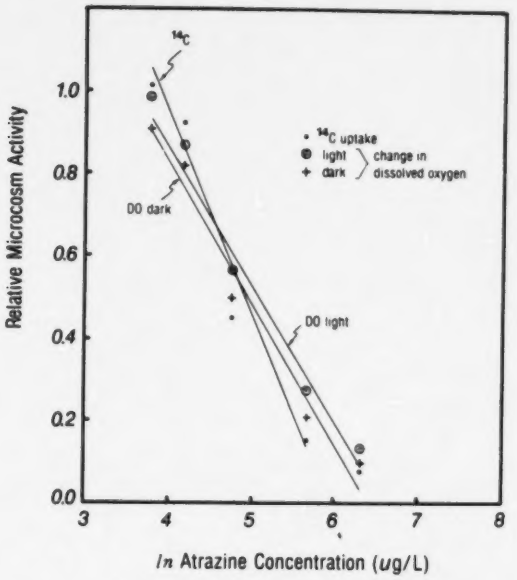

Fig. 1. Dose-response relationships for ^{14}C uptake, dissolved oxygen (DO) production (12-h light period) and DO consumption (12-h dark period) in Taub microcosms covering the entire experimental period, days 11 to 60. EC50 values are summarized in Table 2.

Community production and respiration. Atrazine concentrations of 200 µg/L, and greater, inhibited increases in DO during the light phase and decreases in DO during the dark phase, relative to controls, throughout the microcosm experiment [24]. At 60 and 100 µg/L exposures, inhibition was less apparent and intermittent, while at 200 µg/L inhibition initially occurred, followed by recovery. Dose-response relationships generated for the same intervals as for ^{14}C uptake indicate lowest EC50 values for the first interval (126 µg/L for the light phase; 106 µg/L for the dark phase) and higher values for the later interval and the entire experimental period (Table 2). See Figure 1 for a typical dose-response relationship. The EC50 values for these two measures are similar to, though slightly higher than, those for ^{14}C uptake.

Ponds

^{14}C uptake. During 1981 there were no clear differences in ^{14}C uptake between controls and 20 µg/L treatments. Significant reductions in ^{14}C uptake occurred at the 100 and 200 µg/L treatment levels [30] (deNoyelles, unpublished observations). Application of atrazine caused an immediate lowering of ^{14}C uptake for about 2 weeks. After this initial 2-week interval, ^{14}C uptake returned to control levels, then decreased relative to the control after another 2 weeks and remained depressed through 4 months after treatment. Then treatments became indistinguishable from controls. A similar pattern of immediate response followed by recovery, then depression, occurred at the 500 µg/L treatment level during 1979; the experiment was not conducted long enough to determine whether ^{14}C uptake at this treatment level returned to control values [26].

The ^{14}C uptake data used for dose-response calculations covered two intervals each year. The first interval was that period immediately following atrazine addition (15 d for 1979; 10 d for 1981) when significant effects were maximum. The second interval covered days 91 to 114 (in 1979) and days 49 to 136 (in 1981) after addition of atrazine, periods during which treatment ponds differed from controls at the higher dosage levels. Time-weighted averages of ^{14}C uptake over each interval were used for the calculations.

To compare inhibition during 1979 and 1981, and to estimate EC50 values based on data from both years, all data were expressed relative to an estimate of the control value. Because of the limited number of ponds available for study, only two ponds were used as true controls each year. During 1979, two ponds were exposed to 20 µg/L atrazine, and during 1981, four were exposed at 20 µg/L atrazine. No clear effects on algal photosynthesis occurred at the 20 µg/L treatment level. As mentioned previously, dose-response relationships were estimated for two intervals, one

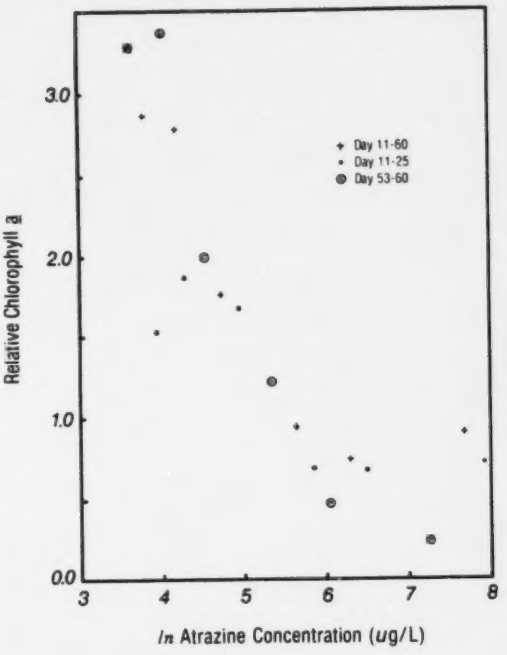

Fig. 2. Chlorophyll *a* relative to control values in Taub microcosms for three time intervals: days 11 to 25, days 53 to 60 and days 11 to 60.

covering the first 10–15 d of the experiment, and the other covering a later interval. During the first interval in 1979, one control value fell between the two 20 μg/L values; the second control value was about 2.5 times greater than the first. During the first interval in 1981, the control values were similar, and three of the four 20 μg/L values were similar to each other and to the control values. The fourth 20 μg/L value was about twice the value of the other three. Therefore, in generating the dose-response relationships, mean values of 0 and 20 μg/L treatments were used to estimate mean control values for each year, excluding the two apparent outliers. The relative value for each pond was then calculated as a fraction, using the mean estimated control value for the respective year as the denominator. All relative values for treatment and control ponds during the first interval are plotted in Figure 3. During the later interval, no control or 20 μg/L treatment values were clearly substantially higher or lower than others, so all values (0 and 20 μg/L) were used to estimate the mean control value for each year.

During both intervals, a well-defined dose-response relationship is evident (Figs. 3 and 4). During the earlier interval, the relationship is better defined and, at higher dose levels, inhibition is more complete than in the later period. However, for both intervals, estimated EC50 values were similar (99 μg/L for the first interval and 101 μg/L for the second interval; Figs. 3 and 4).

Chlorophyll a. During 1981, chlorophyll *a* did not respond in the clear way that ^{14}C uptake did immediately after treatment at the 100 μg/L and higher treatment levels. Similarly, no effects were observed at the 20 μg/L level. Chlorophyll *a* was not measured during the 1979 study.

Longer-term inhibition occurred at the 100 μg/L and higher treatment levels (Fig. 5), but inhibition was not apparent at the 20 μg/L exposure, which is consistent with the observations for ^{14}C uptake. A dose-response relationship was generated for the second interval using the same approach as that for ^{14}C uptake. The estimated EC50 value was 82 μg/L.

24-h inhibition. During 1981 and early 1982, seven 24-h ^{14}C uptake inhibition experiments were conducted by exposing control-pond algal communities to various atrazine concentrations. The data from these experiments form clear dose-response relationships (Fig. 6) from which EC50 values and their 95% confidence intervals were estimated (Table 3). EC50 values ranged from 24 to 131 μg/L.

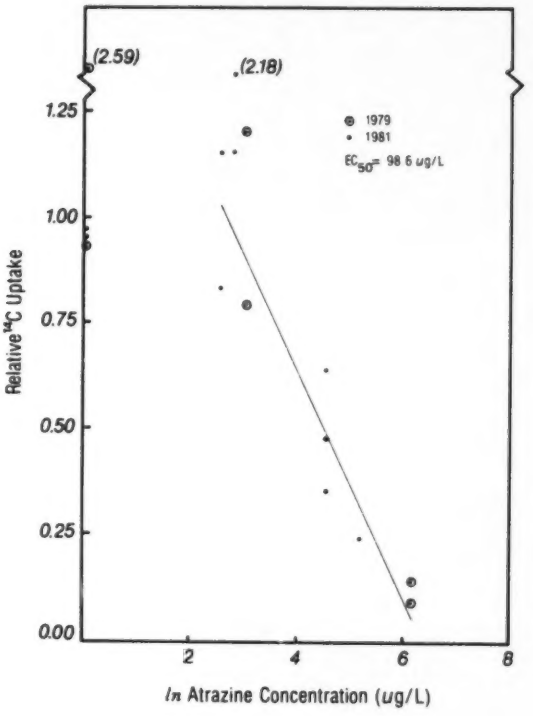

Fig. 3. Dose-response relationship for ^{14}C uptake in Kansas experimental ponds during the initial 15 d after treatment in 1979 and 10 d after treatment in 1981.

Table 3. Estimates of EC50 values using 24-h ^{14}C uptake from Kansas control pond samples exposed to various atrazine doses under laboratory conditions

Date	Days after treatment	LCL (μg/L)	EC50 (μg/L)	UCL (μg/L)
08/04/81	39	17	96	527
08/08/81	43	24	131	729
10/05/81	101	75	109	157
12/20/81	177	23	24	37
03/02/82	249	14	27	40
03/12/82	259	19	37	55
07/04/82	373	94	100	134

LCL and UCL, lower and upper 95% confidence limits, respectively.

DISCUSSION

Comparison of results among the different test systems in this study provides an encouraging picture of the similarity of the concentrations of atrazine that produced direct effects on exposed plants. The concentrations of atrazine that inhibited photosynthesis, respiration and algal biomass development by 50% among the single-species, microcosm and pond studies were generally in the 100 to 150 μg/L range. The single-species algal assays displayed the greatest variation, on the order of 10-fold (Table 1). The short-term (24-h) pond community ^{14}C uptake responses also exhibited significant variation across the days tested, but not nearly as great as that for the single-species assays. Some single-species algal assays and short-term pond community ^{14}C uptake inhibition tests were the most sensitive, with occasional EC50 values of less than 50 μg/L. Also, some single-species algal assays were the least sensitive. The overall similarity in sensitivity of the various test systems probably occurs because atrazine affects the photosynthetic process in all algae in a similar way, by blockage of electron transport in photosystem II [20], although some algae are significantly less sensitive than others. The response of chlorophyll *a* in the microcosms was the only exception to the consistent pattern seen among the other variables.

It is possible that the ponds could have responded in a more sensitive way had the algal community at the time of exposure consisted of more sensitive species, or that it could have responded in a less sensitive way had more resis-

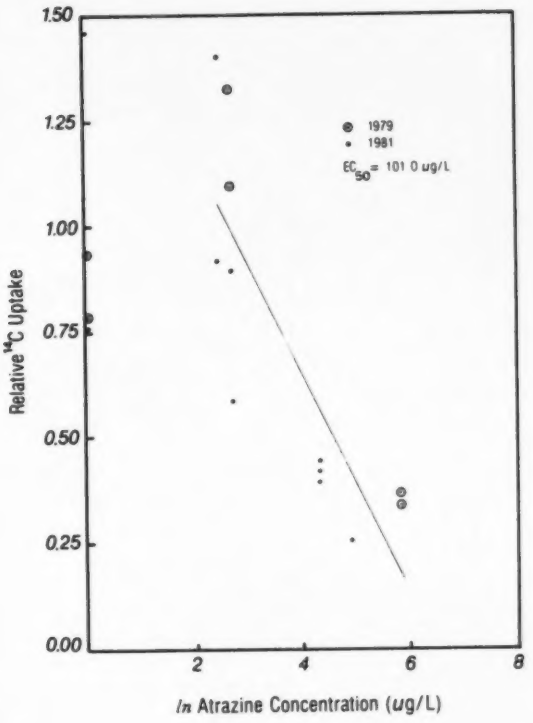

Fig. 4. Dose-response relationship for ^{14}C uptake in Kansas experimental ponds during days 91 to 114 after treatment in 1979 and days 49 to 136 after treatment in 1981.

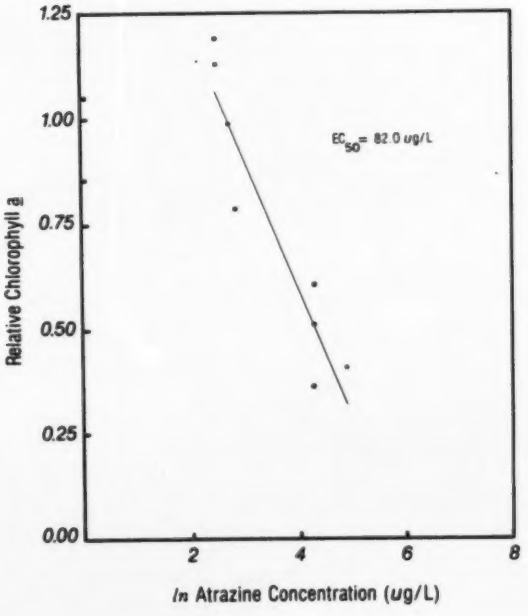

Fig. 5. Dose-response relationship for chlorophyll *a* in Kansas experimental ponds during days 49 to 136 after treatment in 1981.

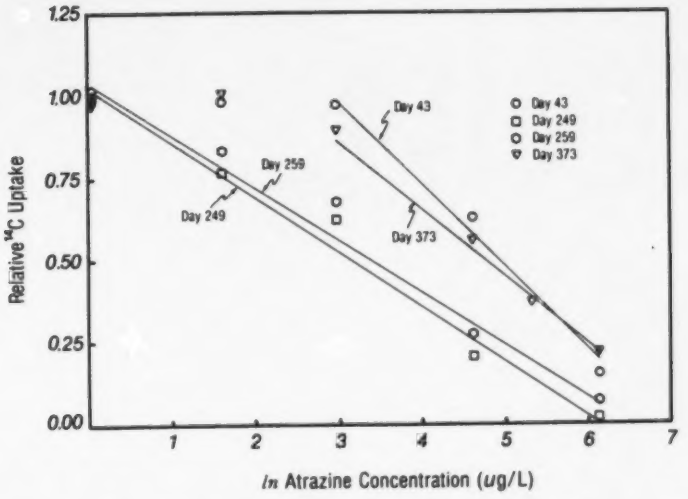

Fig. 6. Dose-response relationships for ^{14}C uptake generated from control-pond algal communities exposed to atrazine for 24 h on various days. To avoid clutter, only 4 of the 7 dose-response relationships are shown; the other relationships were similar. EC50 values are summarized in Table 3.

tant algae been dominant. The short-term pond community inhibition tests provide an estimate of the likely range of sensitivity of the pond communities. Also, pond responses averaged over intervals can reduce apparent sensitivity because some recovery can occur even though treatment pond characteristics are significantly different from those of controls. Similar recovery in microcosms would also cause a decreased apparent sensitivity in the 50% inhibition exposures estimated from microcosm experiments.

The concentrations at which atrazine inhibited various measures of algal activity in this study are similar to those noted in other studies in which no attempt was made to generate dose-response curves. Brockway et al. [31] examined the response of static and flow-through microcosms to atrazine exposures, concluding that negative impacts occurred at and above 50 μg/L. Carney [32] demonstrated that the biomass of macrophyte communities in the Kansas ponds was substantially reduced at atrazine exposures of 100 μg/L. In that study, there appeared to be a differential sensitivity of macrophytes, with *Potamogeton pusillis* and *Najas guadalupensis* affected at the lowest concentrations. *Chara globularis* was least sensitive, but was nearly eliminated by exposure to 500 μg/L atrazine.

Others have established dose-response curves and calculated EC50 values for various algal and macrophyte species. Hollister and Walsh [33] reported a range of sensitivity of from 60 to 460 μg/L atrazine for 18 species of unicellular marine algae based on tests using oxygen evolution as the response variable. These values are consistent with those we estimated for the eight freshwater algal species. Considerable work has been done on aquatic macrophytes, especially those species found in Chesapeake Bay, in which atrazine was suspected to have caused a large-scale decline in macrophyte abundance. The EC50 values for a variety of macrophytes are summarized in Table 4; those estimated for photosynthesis (O_2 production or ^{14}C uptake) are very similar to values calculated in this study. The values estimated for various measures of growth tend to be higher and more variable among species.

Generic toxicity assay systems form the basis for most environmental hazard evaluation systems. Because broad ranges in species sensitivities occur, use of only a few tests species might not offer sufficient environmental protection. In the present study, both generic single-species and microcosm tests produced estimates of effect exposures that are consistent with those seen in experimental pond studies. However, the single-species assays

Table 4. Summary of reported EC50 values for various macrophyte species exposed to atrazine

Source	Macrophyte species	Response variable	Exposure duration	EC50 (μg/L)
Kemp et al. [36]	*Potamogeton perfoliatus*	Photosynthesis (O_2 production)	4 weeks, static microcosms	55
	Myriophyllum spicatum	Photosynthesis (O_2 production)	4 weeks, static microcosms	117
Jones et al. [37]	*P. perfoliatus*	Photosynthesis (^{14}C uptake)	24 h	80
Jones and Winchell [38]	*P. perfoliatus*	Photosynthesis (^{14}C uptake)	24 h	77
	M. spicatum	Photosynthesis (^{14}C uptake)	24 h	104
	Zannichellia palustris	Photosynthesis (^{14}C uptake)	24 h	91
	Ruppia maritima	Photosynthesis (^{14}C uptake)	24 h	102
Forney and Davis [39]	*Elodea canadensis*	Growth (stem length increase)	3 weeks, 3.8-L glass jars	80–109 (2 experiments)
	Vallisneria americana	Growth (leaf length)	3 weeks, 3.8-L glass jars	163–532 (2 experiments)
	P. perfoliatus	Growth (length)	3 weeks, 3.8-L glass jars	474
	P. perfoliatus	Growth (dry weight)	3 weeks, 3.8-L glass jars	907
	P. perfoliatus	Percent killed	3 weeks, 3.8-L glass jars	53
	M. spicatum	Growth (length)	3 weeks, 3.8-L glass jars	1,104
Walsh et al. [40]	*Thalassia testudnium*	Oxygen evolution	40-h flow-through systems	320

indicate a broad range of effect exposures, dependent on the sensitivity of the respective algae. This broad range of sensitivities is typical of the among-species variation seen for a great many chemicals [34]. In this study, if the least sensitive algal species were used to estimate exposure concentrations, predictions would have been poor. Improvement in predictive ability occurs when several species are used as test organisms so that a range of effect levels can be estimated. This approach has been adopted to estimate the criterion for the safe exposures to toxic chemicals [34]. However, initial tiers of toxicity testing protocols [3] rely on fewer species, generally only three, one of which is an alga. If broad ranges in sensitivities occur, then the probability for evaluating a chemical as safe, when it is in fact not, increases if only a few species are used in screening tests. A multispecies test, such as a generic microcosm, might provide estimates of ranges of effect exposures more clearly indicative of exposures at which environmental effects might occur. In this study, the Taub microcosm estimated effect exposures, measured as EC50 values, similar to those seen in the ponds. More comparisons between generic test systems and field effect studies using chemicals with differing modes of action are needed to identify the accuracy of generic test systems.

While the comparisons reported herein imply a reasonable consistency among test systems, the comparison is limited in that only selected characteristics of the primary-producer community were used. Part of the purpose of the pond studies was to explore the ramifications of primary effects on the remainder of the pond community. Do important effects occur in natural systems (simulated by the ponds) at exposures significantly below those at which laboratory tests predict that harmful effects ought to occur? An assessment of the results of the ponds experiments is incomplete. However, some important secondary effects have been seen in the ponds exposed to 100 μg/L atrazine and higher. These include changes in phytoplankton community composition as less sensitive species replaced the more sensitive species [30]; reduction in grass carp in response to reduced macrophytes (deNoyelles, manuscript in preparation); changes in insect community structure [35]; and changes in

bluegill growth (deNoyelles, manuscript in preparation). Some of these effects were also detectable in the 20 μg/L ponds, but the effects were not substantial.

Although the secondary effects observed in the pond systems could not necessarily be predicted or deduced from the generic assays, the exposure concentrations at which they occurred did not differ significantly from exposures that the laboratory assays implied should produce significant effects in natural systems. Comparisons among tests for a common endpoint (EC50) demonstrated a similarity of response among the various test systems. We conclude that the array of single-species algal assays and the Taub microcosm predicted atrazine exposure concentrations at which similar effects occurred in pond communities.

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